Changes in histamine levels in dried fish under different storage conditions

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Abstract

Dried queen fish (*Chorinemus sp.*), skipjack tuna (*Katsuwonus pelamis*), herring (*Amblygaster sirm*) and anchovy (*Anchoviella indica*), obtained from Trincomalee and Kalpitiya processing sites, were stored under different conditions and investigated for the rates of histamine formation over a period of time. Air conditioning (21±2 °C, 72.5±2.5 %RH; representing supermarket conditions) and ambient storage (29±3 °C, 79±7 %RH; representing retail shop conditions) were used as the test conditions with storage at ambient temperature (29±3 °C, 79±7 %RH) after vacuum packing as the control. Samples were analysed for total solids, water activity, salt and histamine levels at two week intervals over a period of two months.

The correlation between water activity (a_w) and changes of histamine (+0.093) levels indicated that the growth of histamine producing bacteria increased with high a_w. The correlation between salt and histamine levels (–0.063) indicated that the histamine production decreased with increase in the salt content, showing a preservative action. Vacuum packing (histamine = 289.2 mg/100 g) was a better storage method, in relation to histamine content when compared with air conditioning (histamine = 323.4 mg/100 g) and ambient storage (histamine = 367.2 mg/100 g). The growth of facultative anaerobic histamine producing bacteria (HPB) may be the reason for the histamine formation in vacuum packed dried fish whereas in the other two storage conditions, mesophilic HPB may be the cause. Species-specific effect on the development of histamine levels was evident from the findings that the values in queen fish (11.1 mg/100 g) and anchovy (39.7 mg/100 g) were significantly lower ($P<0.05$) than in herring (152.1 mg/100 g) and skipjack tuna (1103.5 mg/100 g). Results show that formation of histamine during storage is affected by salt content, water activity (a_w), initial quality of dried fish, temperature and Relative Humidity.

Keywords: histamine, dried fish, storage, water activity, salt

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Introduction

Dried fish is popular among Sri Lankans even though there are a number of fresh fish and fish products available in the market. Dried fish processing in Sri Lanka is a small-scale industry, where the moisture content of the fish is decreased by salting followed by drying or smoking. There is a belief among fishermen that this process is also suitable for fish that is partially spoilt (Fonseka *et al.*, 1995).

Some people exhibit immediate and intense reactions while others can exhibit milder responses when certain types of dried fish types are consumed. Histamine is often the causative agent for these reactions with the condition being described as histamine intoxication. Ingestion of food containing small amounts of histamine has little effect on humans, but in larger amounts, histamine can be toxic (Kerr *et al.*, 2002). The intestinal tract of humans produces the enzymes diamine oxidase (DAO) and histamine N-methyl transferase (HMT), which convert histamine to harmless products. For a large dose of histamine, however, the capacity of DAO and HMT is insufficient to detoxify histamine, resulting in toxic effects as histamine enters the blood stream (Tayler, 1986). Symptoms of histamine toxicity include vomiting, diarrhoea, abdominal cramps, perspiration, flushing, headaches and a burning sensation in mouth (Taylor and Bush, 1988 quoted by Kerr *et al.*, 2002).

Histamine is a member of a group of compounds known as biogenic amines. These are biologically active compounds normally produced by decarboxylation of free amino acids (Kerr *et al.*, 2002). Histamine is produced by the decarboxylation of histidine by bacteria capable of producing histidine decarboxylase enzyme which has an optimum temperature of 25 °C (Silva *et al.*, 1998). The formation of histamine is dependent upon the availability of free histidine, presence of decarboxylase producing microorganisms and conditions that favour bacterial growth and enzyme processes (Kim *et al.*, 2002, Gunaratne, 1994; Wei *et al.*, 1990).

Materials and Methods

Sampling

Four dried fish species popular in Sri Lanka were selected to represent grades A, B and C classified according to their length by the SLSI (1984). Accordingly, two species from "grade A" queen fish (*Chorinemus sp.*) and skipjack tuna (*Katsuwonus pelamis*), one from "grade B" herring (*Amblygaster sirm*) and one from "grade C" anchovy

(*Anchoviella indica*) were selected. Samples were collected from two different production stations Trincomalee on the East coast and Kandakuliya, Kalpitiya on the North-West coast in Sri Lanka. The samples of dried fish from each species were packed in polyethylene bags and stored under three different storage conditions; these were, air conditioning (at temperature 21±2 °C and RH of 72.5±2.5 %) similar to super market storage, ambient condition (at temperature 29±3 °C and RH of 79±7 %) representing storage in a retail shop and vacuum packed storage under ambient conditions (at temperature 29±3 °C and RH of 79±7 %) as a control. Samples were analysed every two weeks for a total period of two months.

Analysis for water activity (a_w)

Water activity was measured using an A_w Sprint water activity meter. Scraped samples were placed in small containers and water activity was read directly in the meter. All analysis was performed in triplicate.

Analysis for moisture

The moisture content was determined in an oven using a standard procedure (AOAC, 1998) and the dry matter content was calculated. All analyses were performed in duplicate.

Analysis for salt (NaCl) content

The salt content of the samples were determined as chloride; the ions are precipitated using silver nitrate and the excess silver ions titrated against potassium thiocyanate (AOAC, 1998). All analyses were performed in duplicate.

Analysis for histamine

The histamine content of different dried fish species was determined using a fluorometric method (AOAC, 1998). Histamine was extracted from the dried fish using 75 % methanol in water. The extract was passed through an anion exchange resin to remove potential interfering compounds. Absorption was measured in a spectrofluorophotometer against histamine standards, prepared using a stock solution. All analyses were performed in duplicate.

Statistical analysis

Results were calculated as as mean ± standard deviation of replicated measurements. Changes in histamine levels in the different dried fish species were compared using Analysis of Variance (ANOVA) with a 95 % confidence interval and Least Significant Difference (LSD) tests. Correlation analysis was used to examine the relationships between changes in histamine content with respect to salt and the activity of water in dried fish, respectively.

Results and Discussion

Effect of salt content and water activity on histamine levels

The values for water activity ranged between 0.65 – 0.75 throughout the storage period under all storage conditions. The queen fish had water activity values ranging from 0.71± 0.01 to 0.76±0.01 while skipjack tuna, herring and anchovy had water activity values of 0.71±0.01 to 0.74±0.01, 0.69±0.01 to 0.75±0.01 and 0.67±0.01 to 0.74±0.02, respectively. The relationship between increases of histamine with respect to changes in water activity showed a statistical correlation of +0.093.

The variation of values for salt content showed was not related to the storage period or storage condition. As an example, the mean salt percentages for queen fish, skipjack tuna, herring and anchovy purchased from Trincomalee in the air conditioned storage, on the 2nd week had been 14.9 + 0.7, 13.0 + 0.1, 10.3 + 0.1 and 12.3 + 0.5 respectively, while on the 8th week it was 16.1 + 0.2, 12.7 + 0.5, 13.0 + 0.4 and 13.6 + 0.2 respectively. The statistical correlation between changes in histamine content and salt percentage was as –0.063.

The variation of histamine content with time and between species of dried fish

The histamine content of all the samples analysed in the present study increased during the storage. Skipjack tuna (Fig. 1 to 4) showed a higher histamine content than the other studied species. The samples from Trincomalee kept under air conditioning (Fig. 1), had the highest increase in histamine content, from 641.1±0.1 to 1328.1±0.1 mg/100 g. In herring, the histamine content increased from 16.38±0.01 to 231.2±0.01 mg/100 g. In queen fish, the rate of increase of histamine content was low, from 0.1±0.1 to 19.4±0.1 mg/100 g, while for the anchovy it was from 0.1±0.1 to 76.4±0.1 mg/100 g. These increasing trends were found under all storage conditions, the only difference noted being in the amount of histamine (Fig. 1 – 3).

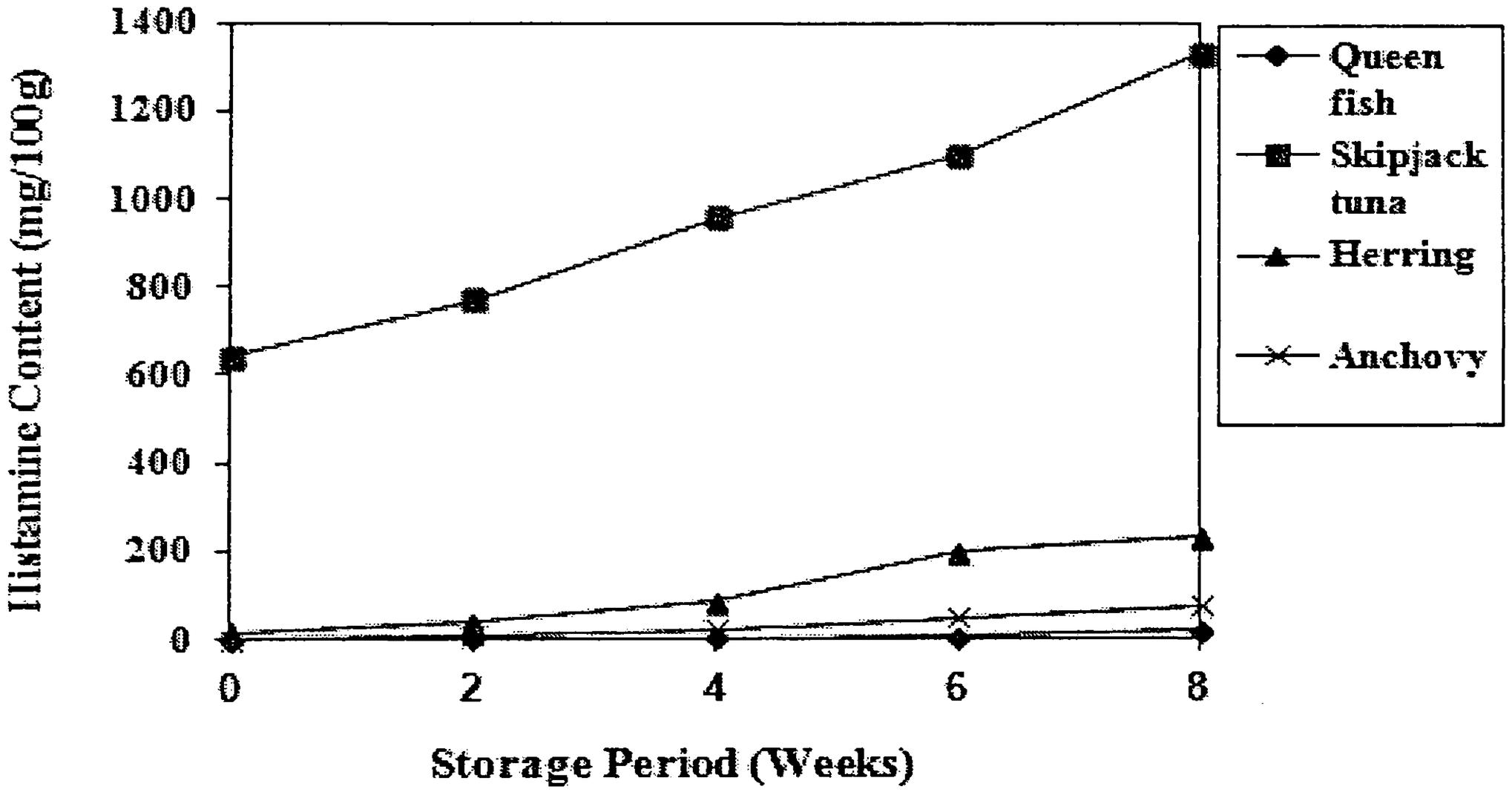

Fig. 1. The histamine production in dried fish from Trincomalee under Air conditioned storage.

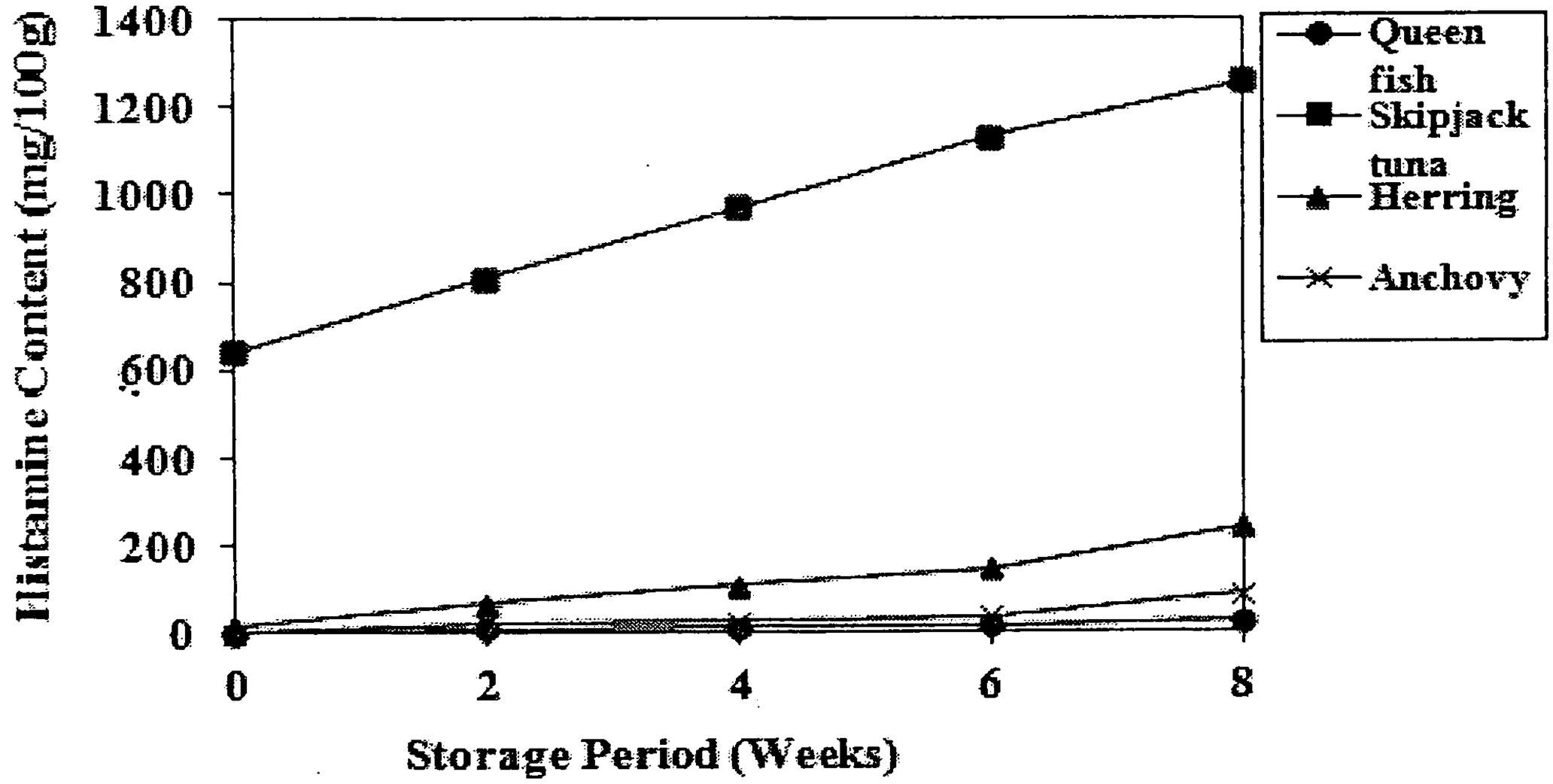

Fig. 2. Time related histamine production in dried fish from Trincomalee under ambient storage conditions.

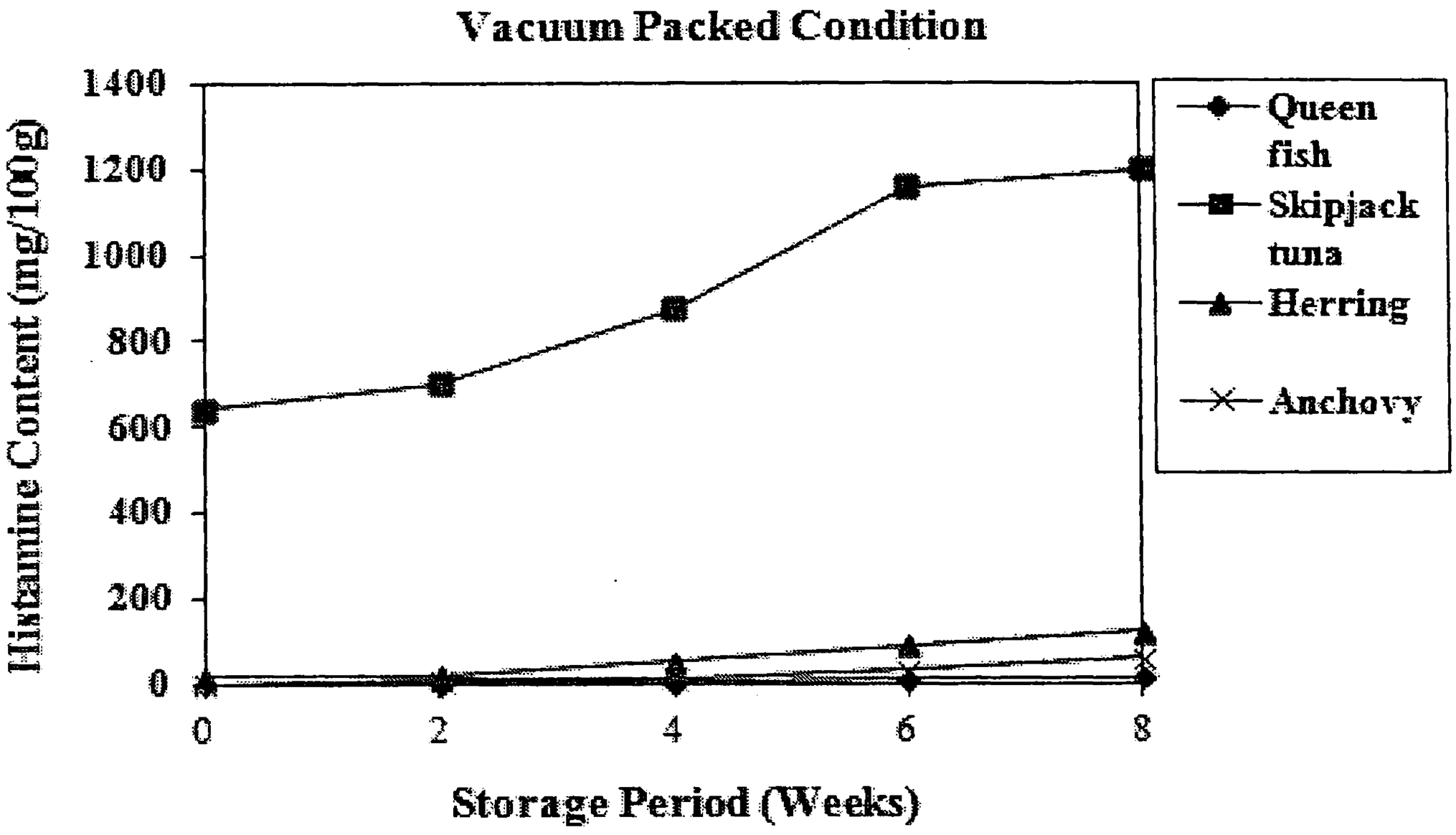

Fig. 3. Time-related histamine production in dried fish from Trincomalee kept under vacuum packed conditions.

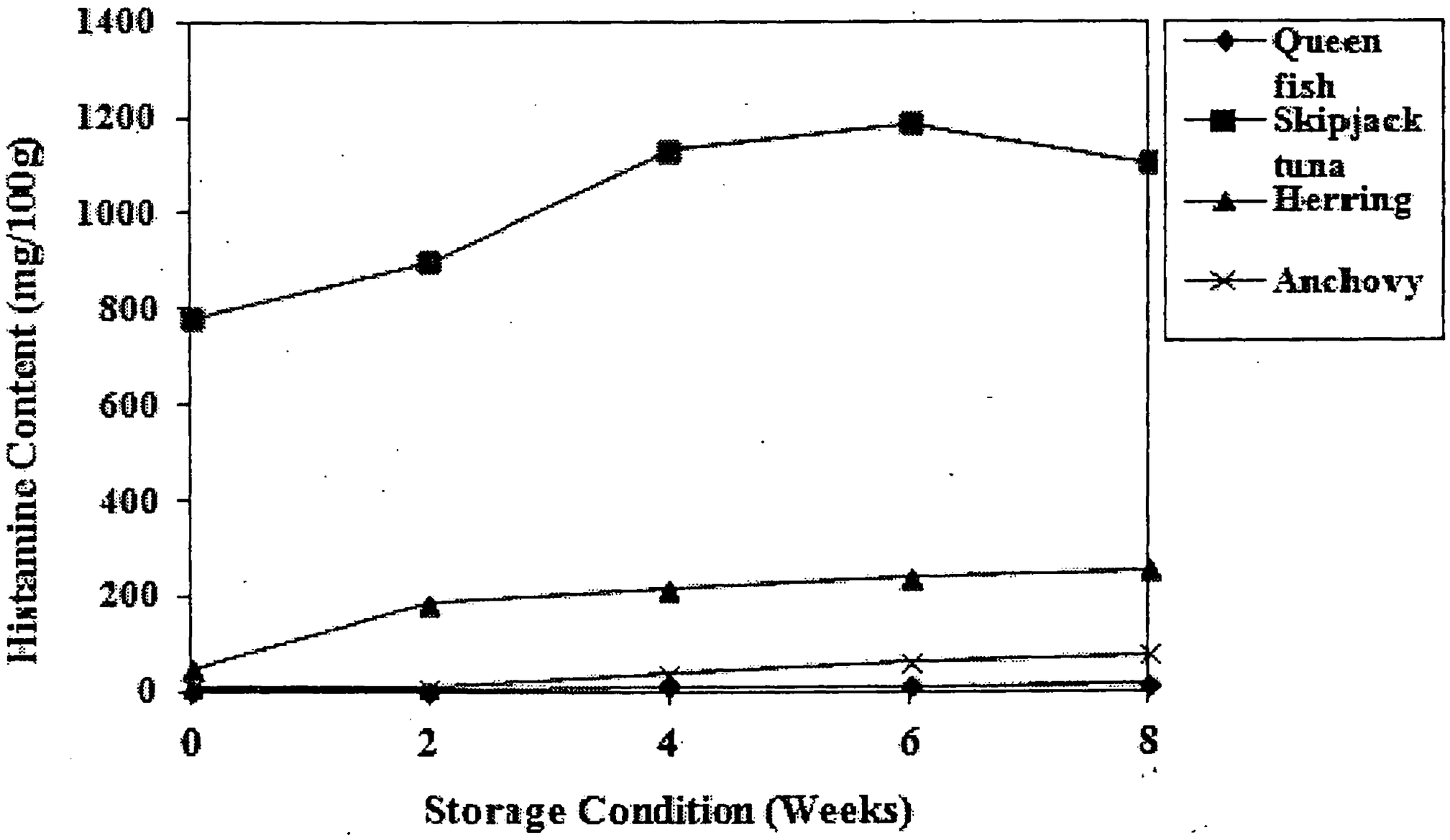

Fig. 4. The histamine production in dried fish from Kalpitiya under air conditioned storage.

The histamine production rates in samples collected from Kalpitiya were generally higher than those from Trincomalee under all three storage conditions (Fig. 4 to 6). The queen fish and anchovy showed similar variation of histamine content in both Kalpitiya and Trincomalee. The initial histamine contents of the samples (producer) of herring, queen fish and anchovy used for the study from both Kalpitiya and Trincomalee were low compared to the skipjack tuna. In both Trincomalee and Kalpitiya, initial queen fish samples had a very low histamine content compared to other species.

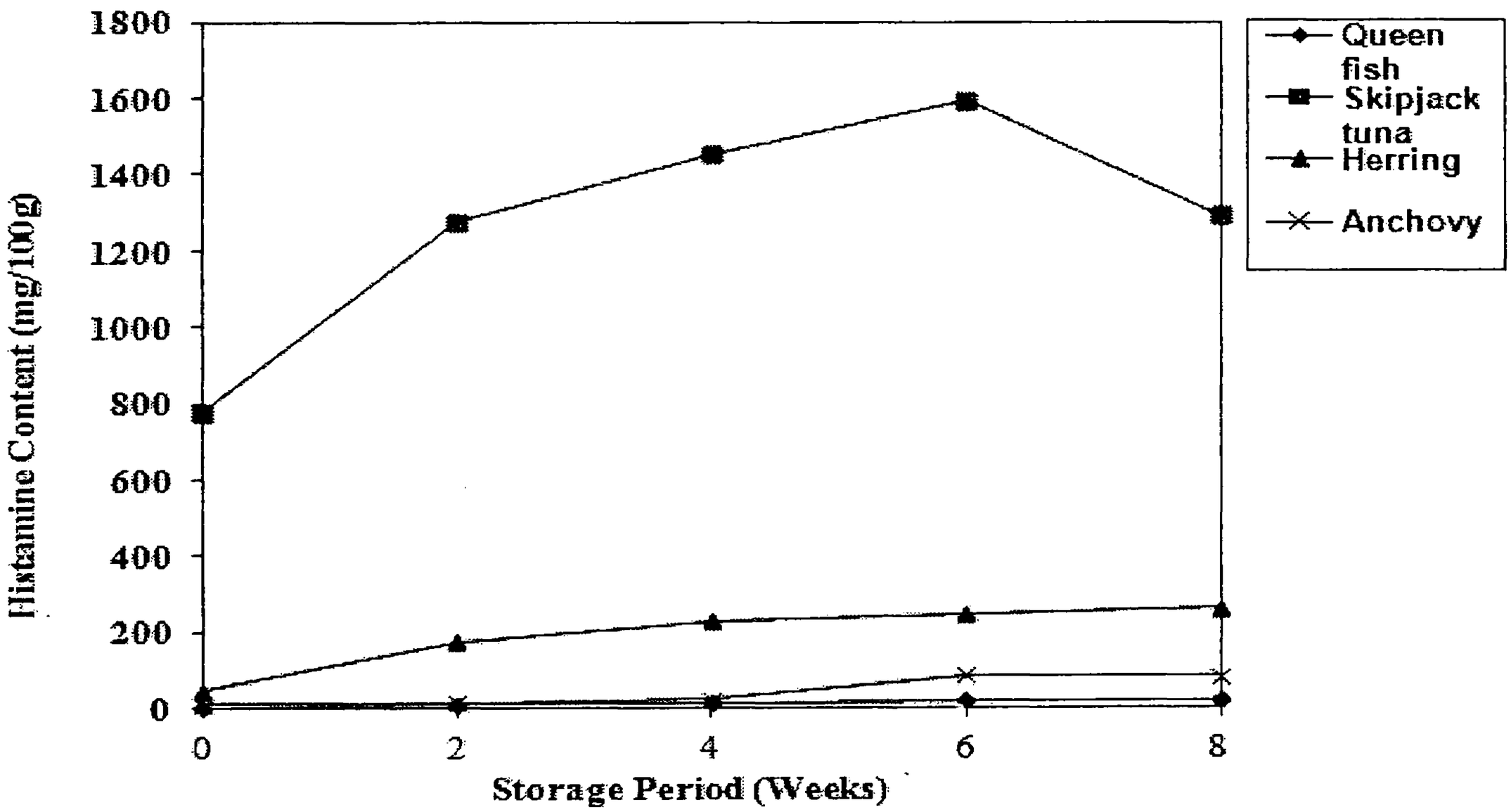

Fig. 5. Time related histamine production in dried fish from Kalpitiya kept under ambient conditions.

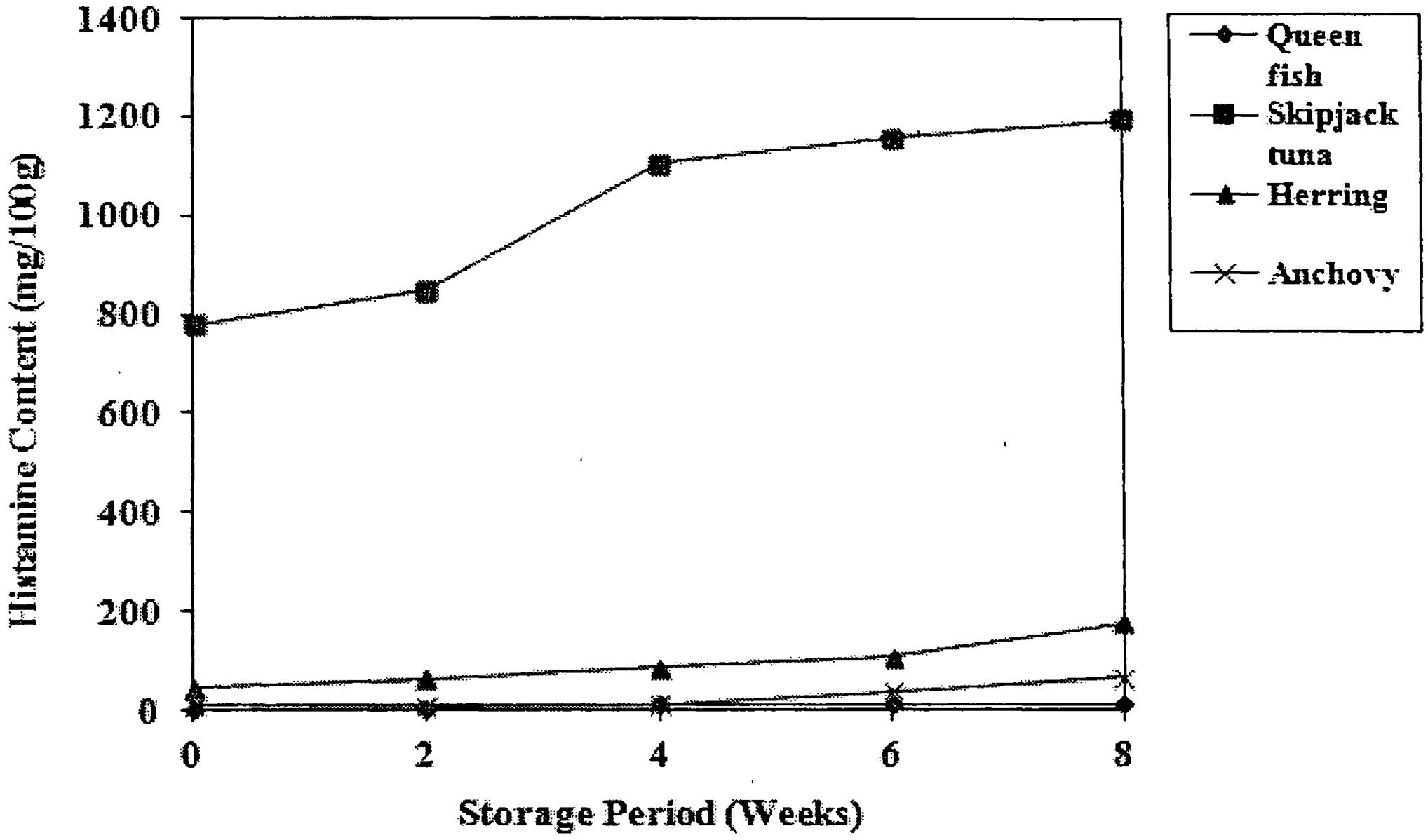

Fig. 6. Time related histamine production in dried fish from Kalpitiya stored under vacuum packed conditions.

The effect of different storage conditions on the development of histamine levels was determined by pooling histamine levels of all fish types. According to the results, histamine production under ambient conditions was significantly different ($P<0.05$) from the control (vacuum packed) (Fig. 7). But samples under air conditioned storage did not differ significantly ($P<0.05$) either from retailed storage or vacuum packed storage. Vacuum packed storage had mean histamine content of 289.16±0.01 mg/100 g, which was the lowest, when compared to air conditioned or ambient storage (323.40±0.01 mg/100 g and 367.23±0.01 mg/100 g), respectively.

The variation of histamine content in different species

Differences between species of fish in the development of histamine were determined by pooling all the data for each species (Fig.8). It can be seen that skipjack tuna had the highest levels followed by herring, anchovy and queen fish. The highest mean histamine content of 1103.5±0.01 mg/100 g, in skipjack tuna was much higher than the those for herring, anchovy and queen fish, which were 152.11±0.01 mg/100 g, 39.70±0.01 mg/100 g and 11.08±0.01 mg/100 g, respectively. The levels of histamine in the dried fish from skipjack tuna was significantly different ($P<0.05$) from the other three. The levels for dried fish of herring was significantly higher than in the other two species but there was no difference between the other two species ($P< 0.05$).

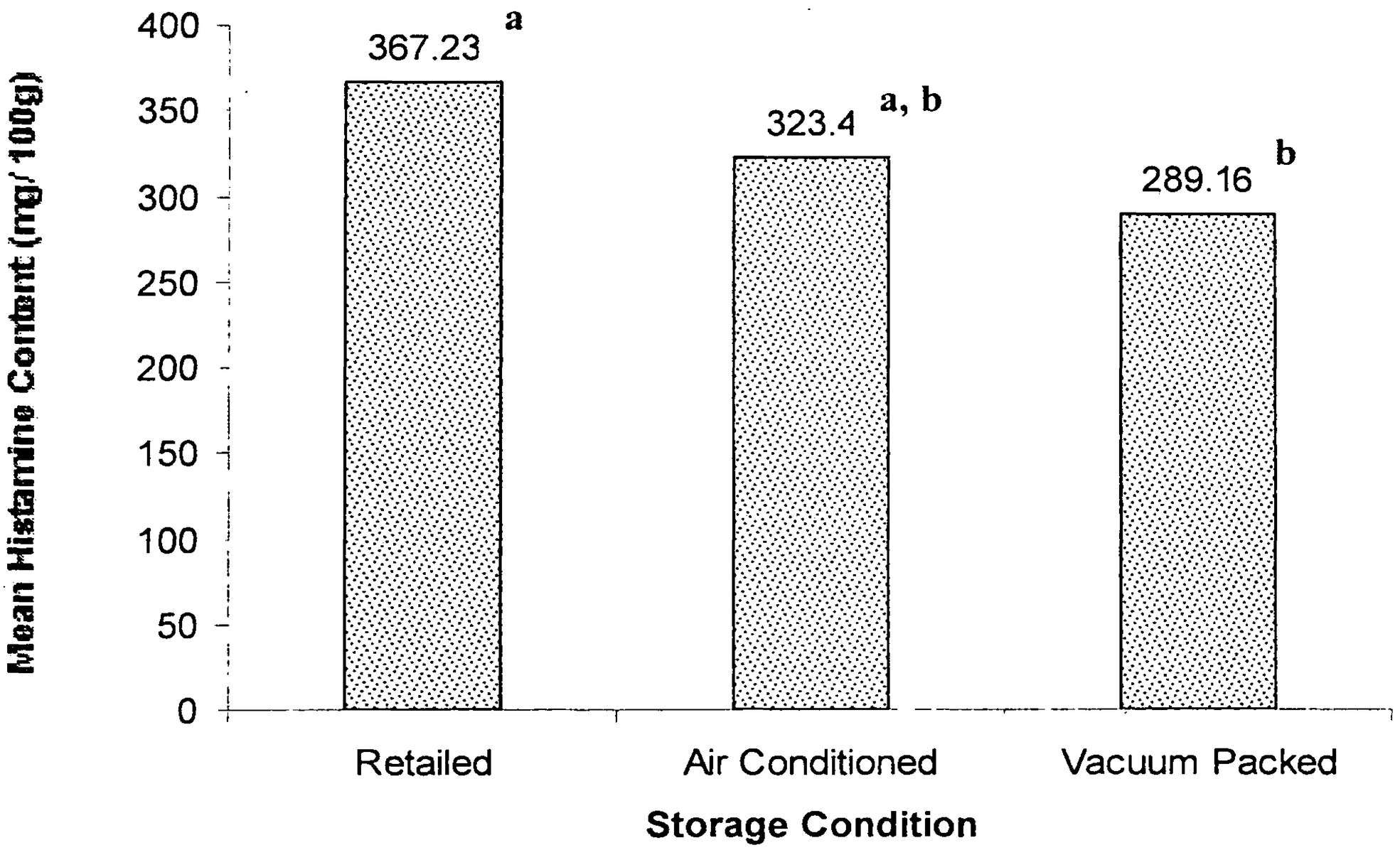

Fig. 7. Mean histamine content of all dried fish samples kept under different storage conditions (Means with different superscripts are significantly (<0.05) different).

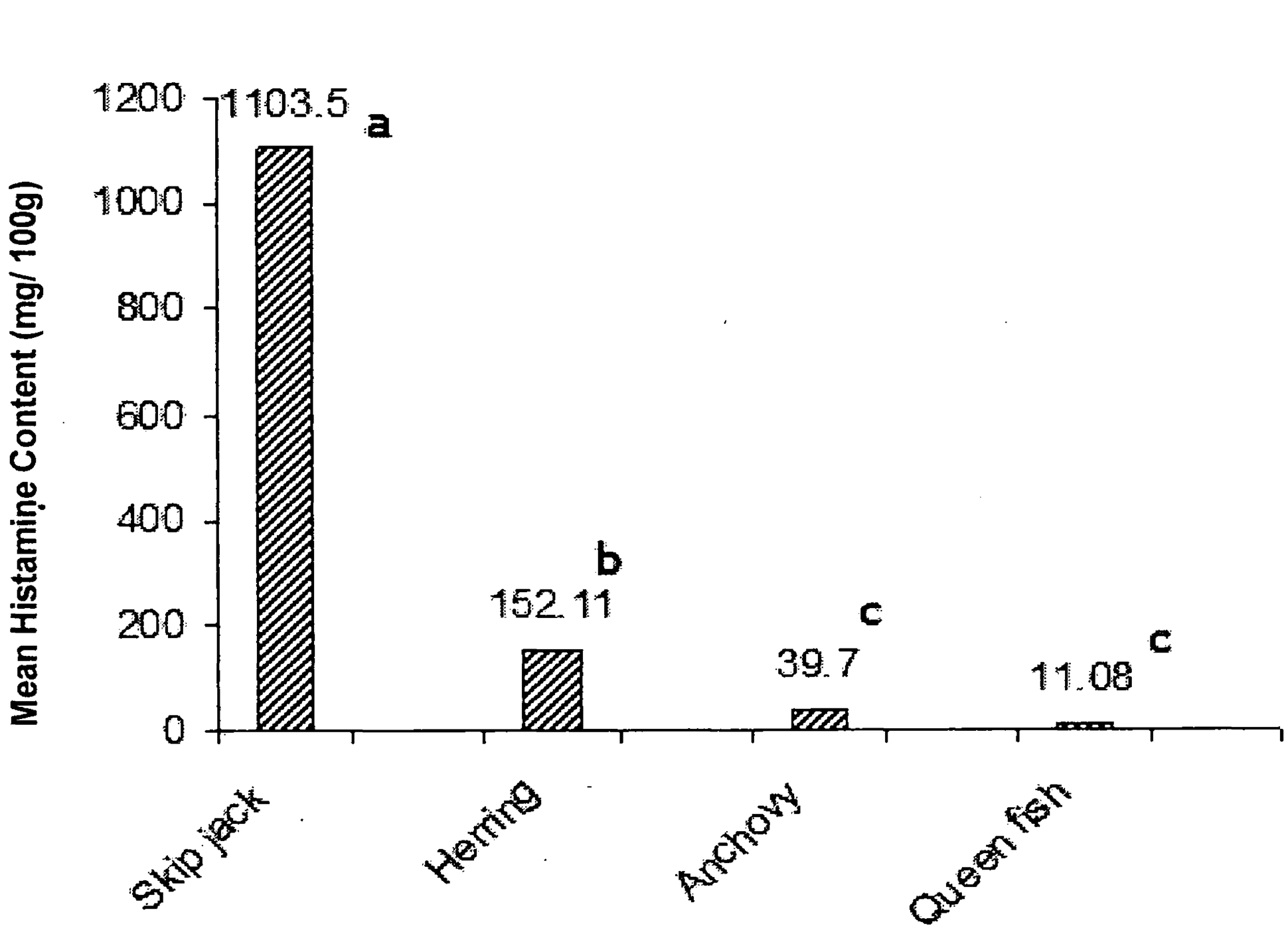

Fig. 8. Mean histamine content in the dried fish of the different species (Means with different superscripts are significantly (< 0.05) different).

Effect of salt percentage and water activity on histamine

Water activity is an indicator of free or available water in food to participate in reactions and a high water activity indicates susceptibility for chemical or microbial spoilage. The correlation coefficient for the increase of histamine with respect to water activity was +0.093. The positive relationship indicates that the histamine levels increased with increases in water activity, since free water was available for the proliferation of histamine producing bacteria.

In the preparation of dried fish, salt is usually used as a preservative where it reduces the water activity by binding water chemically and by causing plasmolysis of microbial cells. The correlation for the increase in histamine levels with respect to salt content was –0.063. The negative sign indicates an inverse relationship where increase of salt concentration decreases the histamine production and shows the preservative action of salt. In both cases, however, the correlations were not strong, being closer to zero. The results on the change of histamine with respect to salt percentage and water activity were in accordance with that of Goonewardene *et al.*, (1980), where they found that the salt used in dried fish preparation in Sri Lanka contains hygroscopic impurities such as calcium and magnesium which take up moisture excessively in the processed products leading to increased in microbial growth.

The variation of histamine content of dried fish species

The main reason for the high content of histamine in other dried fish species compared to queen fish is probably due to the fact that there is no demand for queen fish in the fresh form unlike the other species. Queen fish, therefore, is directly used for dried fish preparation, but the other three species of fish are processed as dried fish only if they were not sold fresh. During this period, the latter are likely to be exposed to high temperatures and cross contamination.

Histamine content of the dried fish under all three storage conditions are above the (Fig. 7), tolerance limits suggested by United States Food and Drug Administration (USFDA) and European Union (EU) for fresh/frozen tuna (5 mg/100 g) and related fish products (10 mg/100 g) (Gunaratne, 1994). The histamine production in after vacuum packed dried fish can be explained as being due to anaerobic or facultatively anaerobic microbial growth, since some studies have shown that there are facultatively anaerobic histamine producing bacteria (Wei *et al.*, 1990, Gunaratne, 1994) that can produce histamine. Most histamine-producing bacteria are mesophiles and the most important factor for

their growth has been temperature (Kim *et al.*, 2002). The temperatures employed in our study were favourable for mesophilic bacterial growth. Also there are mesophilic-halophilic histamine producing bacteria (Yoguchi *et al.*, 1990) and halotolerant histamine producing bacteria (Kim *et al.*, 2001), which are capable of proliferating in the dried fish at temperatures provided.

The variation in histamine content in dried fish made from different species

Many studies have reported a species-specific concentration of histamine (Wei *et al.*, 1990 and Kim *et al.*, 2002). Skipjack tuna showed the highest histamine content as it is a scombroid fish, which contain high levels of histidine (precursor of histamine) in their muscles. Even though herring and anchovy are non-scombroid fish, they have also been implicated previously in histamine intoxication (Gunaratne, 1994); this result agrees with the present experiment.

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